APPLICATION OF THE HIGH-FREQUENCY ELECTRICAL CONDUCTIVITY METHOD FOR THE STUDY OF ADSORPTION PROPERTIES OF IRRADIATEL PROTEINS

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Analysis of the numerous data published in the literature with respect to the action of ionizing radiation on different biochemical systems indicates that disturbances which arise in protein structures play a rather significant role in radiation injury. From this standpoint, the development of sufficiently sensitive physico-chemical and biophysical methods, which will make it possible to detect the earliest and least perceptible physico-chemical disturbances in proteins, must be regarded as one of the most important tasks of radiobiology.

The problem in regard to the investigation of the adsorption properties of protein molecules as a means of characterizing their possible structural modifications has attracted the attention of research workers for a long time. Various methods were applied in this type of investigation [1-6]. A very promising way of investigating the reactivity of proteins proved to be the study of electrical characteristics of protein systems [7-10], particularly with the utilization of the electrical conductivity method. It is worthwhile to note that the methods in question cannot be applied readily because of a number of technical difficulties; it can be seen from the literature that they have not been utilized to a sufficient extent even within the limits of their applicability.

We have developed original procedures for the investigation of the adsorption characteristics of protein solutions. The method devised by us makes it possible to estimate the adsorptive properties of proteins by determining the temperature coefficients of the high-frequency electrical conductivity of their solutions. In the research, which had been carried out and described, the most extensively used method for the investigation of proteins in a wide frequency range (with the predominant utilization of low frequencies) was the bridge method, by means of which the electrical conductivity of a solution being investigated was compared with a standard conductivity. In addition to differences in the design of the equipment, the high-frequency electrical conductivity method developed by us differs from the methods referred to above by the fact that it is based on an investigation of the non-stationary cooling of the solution being investigated [11].

A specific characteristic of this method is its great sensitivity to concentration shifts in the solutions subjected to investigation (the sensitivity of the method is so great that concentrations of the order of 0.0005 N are measured). Furthermore, because of its characteristics, the method makes it possible to investigate reaction properties of proteins in the range of very low electrolyte concentrations and also precludes interaction of protein molecules with the electrodes. This is of great importance because, as has been pointed out by I.Klotz [12], the unavailability of means to bring about these conditions results in technical difficulties in the investigation of the interrelationships between proteins and different ions and thus restricts the application of methods available for this purpose. In addition to these advantages, the method in question is distinguished by its relative simplicity and the speed with which determinations can be carried out by means of it, so that it is of great interest both from the standpoint of application in the investigation of the general problem of the molecular structure of proteins and that of its use in the study of possible structural modifications arising as a result of the action of ionizing radiation.

The principle of the method is as follows (a theoretical basis for the relationships outlined below is given in ref. [11]). The adsorption characteristics of proteins predetermine the different degree of binding by them of various small ions (inorganic, hydroxyl, hydrogen, etc.). An electric field applied from the outside orients the polar molecules in a protein solution and displaces the ions. If the motion of the ions takes place in an alternating field of high frequency (7×10^6 cycles), the large protein molecules cannot follow changes in the field because of their relatively great mass and high inertia. Therefore, the magnitude of the electrical conductivity in this case will be determined solely by the presence of the small ions which surround the protein molecules and interact with them. On the other hand, the adsorption characteristics of the protein molecules (which are manifested either in the freeing or binding of different ions by them) can be characterized on the basis of changes exhibited by the electric conductivity of the solution, because this conductivity depends on the concentration of the free ions which remain in the solution. Thus, if as a result of some action exerted on it the protein in the solution undergoes changes which are accompanied by an increase of its adsorption activity, the concentration of free ions in the solution decreases, and the values of high-frequency electrical conductivity drops to a corresponding extent from their original level. In the case that dissociation or desorption takes place, the concentration of free ions in the solution increases and the high-frequency electrical conductivity rises. At a definite degree of stability of molecular structures, the concentration of small ions in solution does not change in time (within 1-2 hrs.) or changes to only an insignificant extent. The corresponding values of the high-frequency electrical conductivity also remain unchanged in time. In cases like this use was made of relative measure-

ments of the high-frequency electrical conductivity, as an index of the magnitude of which the temperature coefficients of the high-frequency electrical conductivity (TCHE) were employed. Determination of these coefficients made it possible to carry out measurements in a relatively simple and speedy manner, and also with sufficient precision, without resorting to a determination of the absolute values of high-frequency electrical conductivities.

The apparatus for measuring the TCHE of protein solutions consisted of a generator assembled around a 6Zh3P tube which had quartz stabilization (7 megacycles) and a stabilized feedback (electronic stabilization).

The circuit diagram of the equipment is given in Fig. 1, where C_1 and C_2 are calibrated standard capacitors by means of which the oscillating current was tuned to the working frequency (7 megacycles). The capacitance C_1 served as an electrical vernier which made it possible to measure off changes in capacitance with a precision of 0.001 mf. The fact that generation took place was established by means of a control milliampere-meter included into the anode circuit of the generator. A measurement capacitor containing the solution was connected to the terminals of the circuit. A capacitor of this type, in which the concentration of the ionic background of the solution changes, modifies the resonance frequency of the circuit, which is tuned to the required working frequency by the capacitors C_1 and C_2. To carry out the investigation, one must know the value of the capacitance which has been connected to the circuit. This value can be established in a rather simple manner. It is known that if the resonance frequency of an oscillatory circuit is stabilized with quartz placed between the cathode and the grid of the tube, the oscillatory current I_k in the circuit and the constant component of the anode current I_a of the generator tube will change with changes in the capacitance of the circuit as shown in Fig. 2. Generation takes place only in the section AB of capacitance changes. If the capacitance of the circuit is changed near the ends of this section, i.e., in the vicinity of point A, one can find the capacitance value which corresponds to the maximum change in the anode current of the tube (as established by means of the milliampere-meter). It is obvious that connection in parallel to the circuit of some other capacitance changes the found capacitance value which is required for either stopping or starting generation. The difference between the two capacitances required to start or stop generation must give the value of the equivalent capacitance being investigated.

In the case considered by us in this instance, the generator circuit was shunted by means of the complex resistance formed by a test tube containing the solution under investigation. The latter could be regarded as an equivalent capacitance which was connected to the circuit. A change in the value of this capacitance altered the resonance frequency of the current. Investigation of the solutions of

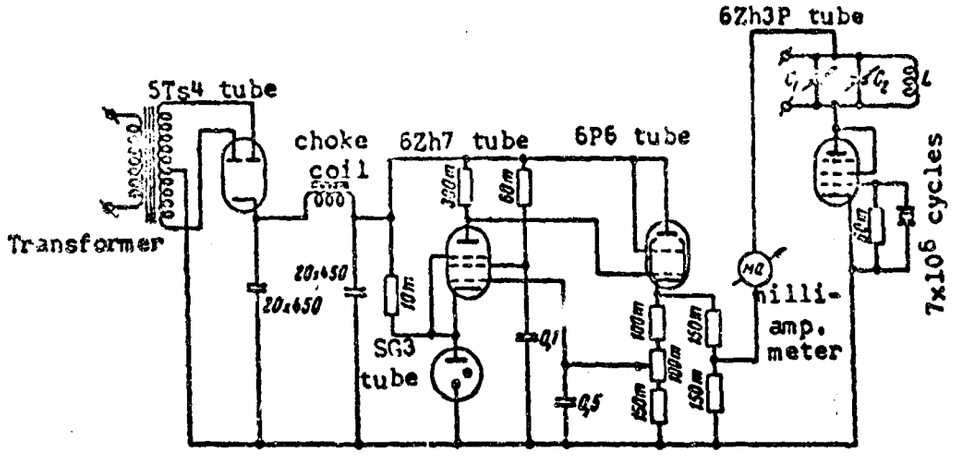

Fig. 1. Diagram of circuit for measuring temperature coefficients of the high-frequency electric conductivity of protein solutions.

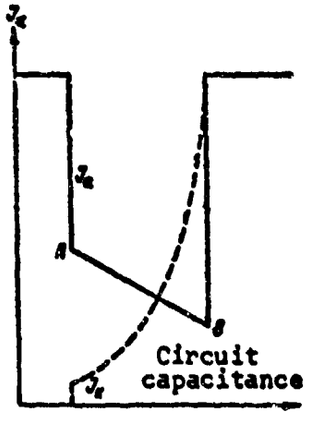

Fig. 2.

interest to us was facilitated by the fact that comparative measurements sufficed.

In view of the fact that the operation of the equipment and the behavior of the solutions being investigated depended on the temperature, the equipment was placed into a constant-temperature chamber,

the daily variation of temperature in which did not exceed 0,2°. The capacitor cell containing the solution was heated by means of a heating sleeve to 37°. The temperature of the solution was measured by means of a thermister sealed into a pyrex glass vial with a wall thickness of 0,1 mm. This vial was filled with polystyrene.

Method Applied in the Investigation.

To carry out experimental measurements, an aqueous protein solution with a definite ionic background was placed into a capacitor cell having the form of a fused quartz test tube with metal coatings on the outer surface of its wall. The test tube was connected to the terminals of the generator. Determinations of the high-frequency electric conductivity were carried out 15, 45, and 90 minutes after the solution had been diluted. They were made in the temperature range of 34-37° with readings taken at every 0,5°. The values of high-temperature electric conductivity obtained at different temperatures (35-36°) could be represented in the form of their interrelationship as the TCHE and calculated graphically.

To determine the number of ions bound under the conditions in question by one molecule of protein, a standard graph representing the relation between the TCHE values and the concentration for the substance under consideration was first constructed. From the known concentration of the reacting molecules, the number of ions bound by the protein could be calculated. If the molecular weight of the protein was known, adsorption could then be expressed in moles of bound ion per mole of protein.

The results of an experiment of this type are shown in Fig. 3. In this typical case, the interaction between cupric ions and the albumin of human plasma was investigated. All standard graphs of this kind should be constructed on the same scale. To the tangent of the angle of inclination of the straight line of such graphs (tg α) corresponded a value equal to the ratio of TCHE values expressed in arbitrary units accepted by us to values of the concentration of the electrolyte in question. For instance, in the case of the $CuCl_2$ solution, tg α was equal to 425×10^3. In calculating the magnitude of adsorption, this value of tg α was used.

The investigation of the binding of copper ions by the protein was conducted in the following manner. To 0,4 ml. of a 1% aqueous human plasma albumin solution, which had been dialyzed for 1.5 hours and which had a TCHE value equal to zero (as has been pointed out above, protein to which no ions had been added and which contained no impurities did not exhibit a high-frequency electric conductivity under the conditions applied by us), 0.2 ml. of an 0,1 N solution of $CuCl_2$ were added. To establish the optimum p_H value of the solution (4.2), 2.2 ml. of an 0,02 M acetate buffer solution were added. The amount of

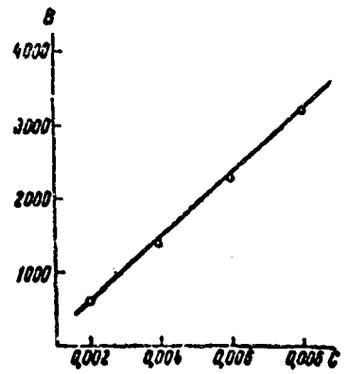

Fig. 3. Standard graph expressing the relation between concentration and the temperature coefficients of high-frequency electrical conductivity for a $CuCl_2$ solution.

On the axis of abcissae, the concentration (C) is plotted in units of normal concentration; on the axis of ordinates, TCHE values (B) are plotted for a $CuCl_2$ solution in arbitrary units.

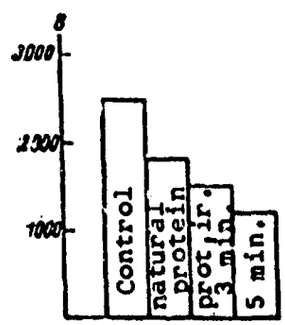

Fig. 4. Changes in the adsorption activity of protein with respect to a $CuCl_2$ solution as a result of the action of γ-radiation.

protein was determined by Weichselbaum's variant of the biuret method [13] and the p_H potentiometrically on an Orion potentiometer with a glass electrode. The total volume of the solution was brought to 0.5 ml. by dilution with twice-distilled water. To the control solution a corresponding quantity of twice-distilled water (0.4) was added instead of the protein. As can be seen in Fig. 4., the quantity of free copper ions was much lower in the solution containing protein than in the control solution (because the TCHE value of the buffer was the same for the experimental solution containing protein as for the control solution, we neglected this value and did not consider it in the subsequent calculations).

The lowering of the TCHE value obtained for the protein solution indicated that the adsorption which took place was of the molecular type, because otherwise there would have been a sharp increase in the electric conductivity due to replacement of Cl^- ions with the more mobile hydrogen ions. Consequently the number of Cu^{++} ions A bound to protein molecules could be calculated from the equation

$$A = \frac{(B_1 - B_2) \cdot M}{m \cdot \operatorname{tg} \alpha}, \qquad (1)$$

where B_1 is the TCHE of the control solution, B_2 the TCHE of the solution containing protein, M the molecular weight of the protein being investigated (in this particular case the molecular weight of the protein was determined by the monomolecular layer method [14] and found to be equal to 70,000), tg α a value determined from the standard graph for the electrolyte being investigated (in this case, tg $\alpha = 425 \times 10^3$ for $CuCl_2$ - cf. Fig. 3), and m the quantity of protein contained in 1 liter of the solution and calculated according to the formula

$$m = 10C \frac{Y}{Y_1}, \qquad (2)$$

where C is the concentration of protein in the initial solution, Y the volume of the protein being investigated, and Y_1 the total volume of the solution. In this manner, the number of Cu^{++} ions bound to one mole of human plasma albumin at $p_H = 4.2$ was found to be equal to approximately 20:

$$A = \frac{700 \cdot 70{,}000}{5.6 \cdot 425 \cdot 10^3} = 20 \text{ ions/mole},$$

which is in agreement with data given in the literature [15, 16].

The action of ionizing radiation on the protein changes considerably its behavior towards copper. Irradiation with γ-rays (Co^{60}) applied in doses of 16,000 and 28,000 r was carried out on a GUT-400 installation with a dose rate of 5.6×10^3 r/min at room temperature.

As can be seen from Fig. 4, the high-frequency electrical conductivity of the protein solution decreased considerably within 5 minutes after the beginning of irradiation, which indicates that the adsorption activity of the protein increased. Furthermore, whereas in the case of the lower dose it was found that

$$A = \frac{1000 \cdot 70{,}000}{5{,}6 \cdot 425 \cdot 10^3} \approx 29 \text{ ions/mole (at } B_2 = 1500 \text{ arbitrary units)},$$

the adsorption was much more pronounced after irradiation with the higher dose:

$$A = \frac{1300 \cdot 70{,}000}{5{,}6 \cdot 425 \cdot 10^3} \approx 38 \text{ ions/mole}$$

Taking into consideration that the molecular weight of the protein remained unchanged after irradiation (according to data obtained by the monomolecular layer method), one may assume that the deterioration of the protein molecules in solution under the effect of the γ-radiation doses applied by us consisted in changes of the structure of the protein globules, which were accompanied by the opening of hydrogen bonds that blocked reactive groups of the molecule.

Thus, changes in the capacity of protein to bind cupric ions as a result of action exerted by γ-radiation can be explained by an increased accessibility to these ions of peptide nitrogen atoms [5], which are mainly responsible for the formation of protein-copper complexes.

It would be advantageous, by making use of the selective adsorbability of some inorganic ions with reference to definite reactive groups of the protein molecule, to apply the method described for the investigation of the kinetics of adsorption of different ions by irradiated proteins. Investigations of this type will presumably contribute to an understanding of the nature and mechanism of the damaging effect exerted by ionizing radiation on proteins. The preliminary data obtained indicate that research along this line must be regarded as promising.

Conclusions

1. A method has been developed for the determination of the temperature coefficients of high-frequency electrical conductivity, by means of which subtle physico-chemical changes in protein structures can be detected. By using the method in question, one can investigate the kinetics of adsorption processes on proteins as affected by the action of ionizing radiation.

2. In the work described, quantitative expressions have been derived for the adsorption of cupric ions by one protein mole of human plasma albumin. It was established that natural protein and protein irradiated with different doses of γ-radiation interacted differently

with cupric ions, the results obtained indicating that ionizing radiation exerts a damaging effect on protein macromolecules.

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